# The role of trimetazidine in inhibiting cardiomyocyte apoptosis

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## Abstract

Trimetazidine is a clinically effective cellular anti-ischaemic agent that has no negative inotropic or vasodilator properties. It is conventionally used mainly for patients with coronary or cerebrovascular disease. Recent studies demonstrated that trimetazidine also has an important role in protecting against ischaemia-reperfusion injury by inhibiting cardiomyocyte apoptosis in a rabbit model of ischaemia-reperfusion. The possible mechanisms of trimetazidine in inhibiting cardiomyocyte apoptosis might be due to the combined effects of limiting $Na^+$ and $Ca^{2+}$ accumulation and reducing intracellular acidosis during low-flow simulated ischaemia, improving myocardial energy metabolism and modulating mitochondrial permeability transition during myocardial ischaemia, increasing endogenous antioxidant capacity and protecting against oxygen free radical-induced toxicity, as well as inhibiting neutrophil infiltration and attenuating the myocardial inflammatory reaction. But this remains to be identified definitively.

**Key words:** trimetazidine, apoptosis, myocardium, mechanism.

## Introduction

The study of cell death and its impact on physiological functions and pathological processes has now become an important area of research, not only in cell biology but also in all spheres of biomedicine. Apoptosis is an energy-requiring physiological mechanism of cell deletion that regulates cell mass in many tissues. It is also called programmed cell death because it is a genetically directed process that takes place in response to internal or external stimuli. Cardiomyocyte apoptosis has important pathophysiological consequences contributing to functional abnormalities in the myocardium. It has been reported in a variety of cardiovascular diseases, including myocardial infarction [1-3], end-stage heart failure [4], arrhythmogenic right ventricular dysplasia [5] and doxorubicin-induced cardiomyopathy [6]. Growing evidence from *in vitro* and *in vivo* studies indicates that inhibition of cardiomyocyte apoptosis would minimize cardiac injury induced by myocardial disorder [2, 7-11].

Trimetazidine [1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride] is a well-established anti-ischaemic drug, which has been studied in clinical and experimental investigations, and was shown to have protective effects against myocardial ischaemia and reperfusion injury [12-16]. This observation suggests a possibility that trimetazidine may have anti-apoptotic effects. In two recent studies of a rabbit ischaemia-reperfusion

model, we found that pretreatment with trimetazidine for two weeks significantly decreases cardiomyocyte apoptosis and improves cardiac performance [10, 11]. This article reviews the possible mechanisms of trimetazidine in inhibiting cardiomyocyte apoptosis.

## Effects of trimetazidine on inhibiting cardiomyocyte apoptosis

Our recent studies showed that trimetazidine pretreatment for two weeks significantly decreases cardiomyocyte apoptosis and improves cardiac performance. Thirty male New Zealand White rabbits were randomly divided into sham, control and treated groups. Trimetazidine (2 mg/g×day) was additionally administered for two weeks to treated animals before the procedure. Mean arterial pressure, left ventricular systolic pressure and the maximum rate of left ventricular pressure rise were significantly higher in the treated than in the control group, whereas left ventricular end-diastolic pressure was significantly lower in the treated than in the control group. As compared with the sham group (Figure 1A), the control group (Figure 1B) had a significantly higher apoptotic index and serum malondialdehyde (MDA) concentration, and significantly lower total activity of serum superoxide dismutase (SOD). Trimetazidine pretreatment apparently decreased apoptotic index (Figure 1C) and MDA concentration, and increased SOD levels. Caspase-3 activation and mitochondrial cytochrome c release were also higher in the control than in the treated group. The apoptotic indices were negatively correlated with SOD and positively correlated with MDA in the groups, suggesting that trimetazidine may be an effective drug in preventing cardiomyocyte apoptosis and ischaemia-reperfusion injury [10, 11].

## Possible mechanisms of trimetazidine in inhibiting cardiomyocyte apoptosis

Apoptosis is an evolutionarily conserved, genetically controlled process of programmed cell death, used by multicellular organisms to eliminate cells in diverse physiological settings, such as development, homeostasis of tissues, and maintenance of integrity of the organism. The apoptotic cascade can be triggered through two major pathways. Extracellular signals such as members of the tumour necrosis factor family can activate the receptor-mediated extrinsic pathway (caspase 8). Alternatively, stress signals such as DNA damage, hypoxia and loss of survival signals may trigger the mitochondrial intrinsic pathway. In the latter, mitochondrial damage results in cytochrome c release and formation of the apoptosome, a multimeric protein complex containing apoptotic protease activating factor-1, cytochrome c and pro-caspase-9. Once bound to the apoptosome, pro-caspase-9 is activated, and subsequently triggers a cascade of effector caspase activation and proteolysis, leading to apoptotic cell death. Apoptosis can be influenced by a wide variety of regulatory

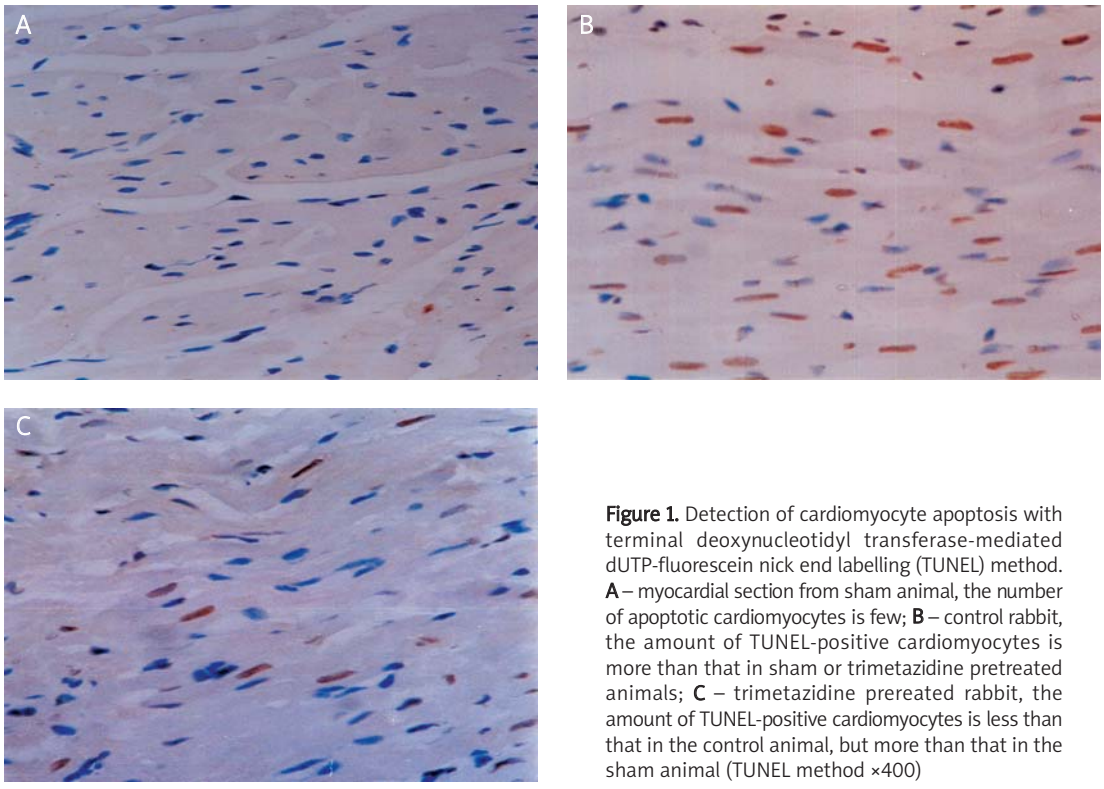


**Figure 1.** Detection of cardiomyocyte apoptosis with terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick end labelling (TUNEL) method. **A** – myocardial section from sham animal, the number of apoptotic cardiomyocytes is few; **B** – control rabbit, the amount of TUNEL-positive cardiomyocytes is more than that in sham or trimetazidine pretreated animals; **C** – trimetazidine prereated rabbit, the amount of TUNEL-positive cardiomyocytes is less than that in the control animal, but more than that in the sham animal (TUNEL method ×400)

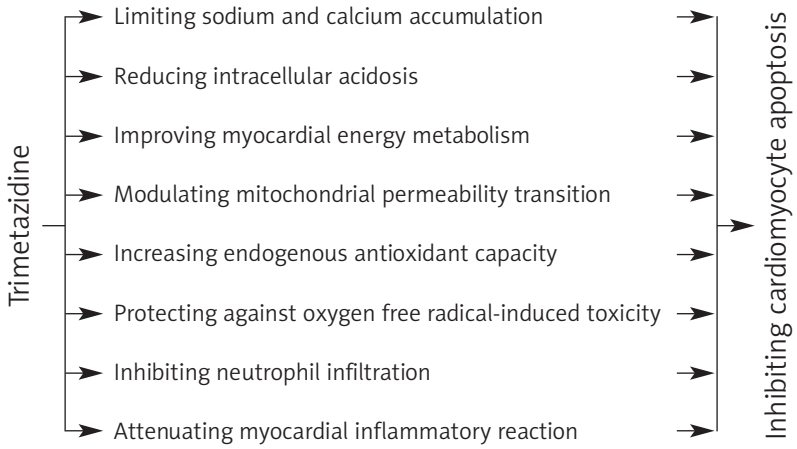


**Figure 2.** Possible mechanisms of trimetazidine in inhibiting cardiomyocyte apoptosis

stimuli. Experimental studies have shown that cardiomyocyte apoptosis is induced during hypoxia, continuous ischaemia or ischaemia followed by reperfusion [17-20]. A specific association with reperfusion injury has even been suggested [19]. The cellular mechanisms underlying both ischaemia-reperfusion injury and apoptosis may involve cellular calcium overload [21], over-production of oxygen-derived free radicals [21], cellular acidosis, inflammatory reaction [21, 22] and microcirculatory dysfunction [23]. In addition, several agents, including catecholamines [24], angiotensin II [25], aldosterone, atrial natriuretic peptide [26], tumour necrosis factor alpha [27], endothelin-1 [28] and cytokines [29] can induce cardiomyocyte apoptosis and necrosis, either in culture or in intact animals. Thus, we can hypothesize that the role of trimetazidine in inhibiting the apoptosis of cardiomyocytes may involve several aspects (Figure 2).

## Trimetazidine limits $Na^+$ and $Ca^{2+}$ accumulation and depresses intracellular acidosis

Dysregulation of intracellular $Ca^{2+}$ homeostasis plays an important role in mediating myocardial injury. A marked increase in cytosolic free calcium has been reported in ischaemic myocardial injury, and the occurrence of intracellular $Ca^{2+}$ overload has been suggested to lead to arrhythmias, contractile failure and ultimately cardiomyocyte apoptosis or death. It has been proposed that trimetazidine plays a key role in limiting the intracellular accumulation of protons that is responsible for cell acidosis during ischaemia. When cardiac cells were kept in normal physiologic conditions, trimetazidine at concentrations ranging from $10^{-8}$ to $3.10^{-4}$ M interacted neither directly nor indirectly with the major ionic transporter systems of cardiac cells, such as ionic channels ($Na^+$, $K^+$), ATPase, $Na^+/H^+$ and $Na^+/Ca^{2+}$ exchange systems. Under acid-load conditions trimetazidine acts in a dose- and time-dependent manner in limiting the accumulation of $Na^+$ and $Ca^{2+}$ inside cardiac cells and depressing intracellular cell acidosis [30]. During low-flow ischaemia (coronary flow decreased by an average of 90%, 30 min at 37°C) the major effect of trimetazidine ($10^{-6}$ M) was a significant reduction in intracellular acidosis, whereas during zero-flow ischaemia the main effect of trimetazidine was a significant reduction in $Na^+$ gain. In addition, the further gain in $Na^+$ that occurred during the first minutes of reperfusion following zero-flow ischaemia, and to a far lesser extent following low-flow ischaemia, was suppressed in trimetazidine-treated hearts and also suppressed when hearts were perfused without fatty acid. In both low-flow ischaemia and zero-flow ischaemia, trimetazidine-induced attenuation of ionic imbalance was associated with significantly improved recovery of ventricular function on reperfusion, as assessed by a lower increase in diastolic pressure and increased recovery of developed pressure. These data provide evidence that specific myocardial metabolic modulation plays a significant role in reducing ionic imbalance during ischaemia and reperfusion [31].

## Trimetazidine maintains intracellular adenosine triphosphate levels

Recent studies showed the existence of an endogenous mechanism of cellular protection against ischaemia. This mechanism was related mainly to cellular liberation of adenosine, a nucleoside with protective effects in myocardial ischaemia. The administration of trimetazidine at doses of 10, 20, 40 and 80 mg in patients with angina pectoris induced an increase of adenosine plasma levels of 19, 50, 62 and 62%, respectively [32], indicating that the activity of trimetazidine could depend, at least in part, on adenosine mediation, and this interaction opens a new interpretation of the drug's anti-ischaemic

effect. In another recent randomized, double-blind, cross-over study to placebo or trimetazidine (20 mg t.i.d.) for two periods of 90 days, Fragasso et al. [33] assessed the effects of trimetazidine on left ventricular phosphocreatine and adenosine triphosphate (PCr/ATP) ratio in patients with heart failure by means of in vivo $^{31}$P-magnetic resonance spectroscopy. The mean cardiac PCr/ATP ratio was 1.35±0.33 with *placebo*, but was increased by 33% to 1.80±0.50 (P=0.03) with trimetazidine, suggesting that the effects of trimetazidine are associated with the preservation of myocardial high-energy phosphate levels.

### Trimetazidine reduces creatine-phosphokinase release

In Langendorff perfused rat hearts, both pre-treatment and treatment protocols with trimetazidine reduce the myocardial damage caused by global ischaemia following reperfusion. The MB isoenzyme of creatine kinase (CK-MB) and troponin T (cTnT) levels indicated less enzymatic damage in trimetazidine treated hearts during reperfusion [34]. In patients with acute anterior myocardial infarction, trimetazidine pretreatment (40 mg orally about 15 minute before thrombolysis and, subsequently, 20 mg every 8 hours) can decrease creatine kinase normalization time, suggesting that trimetazidine probably reduces reperfusion damage and/or infarct size in patients with anterior acute myocardial infarction subjected to thrombolysis and affects remodelling after myocardial infarction [35].

### Trimetazidine preserves mitochondrial functions

Mitochondria are key factors in energy production in cells. They are also key factors in their life cycle because under certain circumstances they can provoke cellular apoptosis. Some 45 per cent of myocardial volume is taken up by mitochondria. Furthermore, mitochondria are key to many aspects of neuronal activity and can trigger neurodegenerative processes. Lipid oxidation is responsible for the production of much ATP resynthesis in the heart but this process is less oxygen efficient than glucose oxidation. During ischaemia, lipid oxidation is suddenly blocked, but markedly increased during reperfusion, causing accumulation of potentially toxic metabolites (acylcarnitines, acyl-coenzyme A, lysophospholipids). Trimetazidine inhibits the production of deleterious lipid metabolites [36]. Using a Langendorff rat heart model, Freude et al. [37] examined the effects of trimetazidine on the mitochondrial damage following 30 minutes of ischaemia and 5 minutes of reperfusion. $10^{-3}$ M trimetazidine significantly decreased the glutamate-malate rate in mitochondria from normoxic hearts, and this rate was not further decreased following ischaemia-reperfusion, and $10^{-3}$ M trimetazidine also partially protected ascorbate-N,N,N',N'-tetramethylphenylenediamine (TMPD) activity. The effect on glutamate-malate was probably due to inhibition of complex I by trimetazidine, which specifically inhibited reduced nicotinamide-adenine-dinucleotide-cytochrome c reductase and complex I in lysed mitochondria. Preperfusion with $10^{-5}$ M trimetazidine had a tendency to decrease lactate dehydrogenase release, accompanied by maintenance of the inhibition of pyruvate dehydrogenase (PDH) by palmitate. Trimetazidine can also inhibit mitochondrial permeability transition pore (mPTP) opening and protects the rabbit heart from prolonged ischaemia-reperfusion injury [38].

### Trimetazidine reduces myocardial fatty acid metabolism

The mechanisms of the adverse effects of free fatty acids on the ischaemic-reperfused myocardium are not fully understood. Long-chain fatty acids, including palmitate, uncouple oxidative phosphorylation and should therefore promote the formation of oxygen-derived free radicals, with consequent adverse effects. Conversely, the anti-anginal agent trimetazidine, known to inhibit cardiac fatty acid oxidation, could hypothetically lessen the formation of reactive oxygen species (ROS) and thus improve reperfusion mechanical function. Isolated perfused rat hearts underwent 30 min of total global ischaemia followed by 30 min of reperfusion. Hearts were perfused with glucose 5.5 mmol/L or palmitate 1.5 mmol/L with or without trimetazidine (100 µmol/L). Trimetazidine, a potential inhibitor of palmitate-induced mitochondrial uncoupling, decreased the formation of free radicals and improved post-ischaemic mechanical dysfunction. The novel conclusion is that adverse effects of fatty acids on ischaemic-reperfusion injury may be mediated, at least in part, by oxygen-derived free radicals [39].

### Trimetazidine increases myocardial glucose metabolism

Trimetazidine has an inhibitory effect on the mitochondrial long-chain 3-ketoacyl coenzyme A thiolase, which plays a critical role in the fatty acid beta-oxidation pathway in the myocardium [40, 41]. As a result, there is a switch of cardiac metabolism from free fatty acid to glucose oxidation, which represents a more efficient metabolic pathway in terms of oxygen consumption and energy (adenosine triphosphate) generation [42]. Trimetazidine increases total glucose utilization (oxidative and glycolytic) in the myocardium without preferential increase in ischaemic tissue. Absence of change in total oxidative metabolism suggests increased glucose metabolism is predominantly glycolysis or an increase in glucose oxidation with similar decrease in fatty acid oxidation [42]. In hearts

subjected to low-flow ischaemia, trimetazidine resulted in a 210% increase in glucose oxidation rates. In both aerobic and ischaemic hearts, glycolytic rates were unaltered by trimetazidine. The effects of trimetazidine on glucose oxidation were accompanied by a 37% increase in the active form of pyruvate dehydrogenase, the rate-limiting enzyme for glucose oxidation. No effect of trimetazidine was observed on glycolysis, glucose oxidation, fatty acid oxidation or active pyruvate dehydrogenase when palmitate was substituted with 0.8 mmol/L octanoate or 1.6 mmol/L butyrate, suggesting that trimetazidine directly inhibits long-chain fatty acid oxidation. This reduction in fatty acid oxidation was accompanied by a significant decrease in the activity of the long-chain isoform of the last enzyme involved in fatty acid beta-oxidation, 3-ketoacyl coenzyme A thiolase activity. In contrast, concentrations of trimetazidine in excess of 10 and 100 µmol/L were needed to inhibit the medium- and short-chain forms of 3-ketoacyl coenzyme A thiolase, respectively. Previous studies have shown that inhibition of fatty acid oxidation and stimulation of glucose oxidation can protect the ischaemic heart. Therefore, these data suggest that the anti-anginal effects of trimetazidine may occur because of inhibition of long-chain 3-ketoacyl coenzyme A thiolase activity, which results in a reduction in fatty acid oxidation and a stimulation of glucose oxidation [40].

### Trimetazidine protects against oxygen free-radical-induced toxicity

The effect of trimetazidine on membrane damage induced by oxygen free radicals in red cells was studied in seven healthy volunteers after oral administration [43]. The results showed that the loss of intracellular $K^+$ induced by oxygen free radicals and the membrane content of peroxidated lipids were significantly reduced in red cells collected after the period of treatment. These results indicate a potent antioxidant activity of trimetazidine which could explain its cardioprotective role during ischaemic and reperfusion phases in which oxygen free radicals are generated and probably implicated in the genesis of cardiac cell injury. Trimetazidine at concentrations above 100 µM competed with cytochrome c in scavenging $O_2^{\cdot}$ – radicals formed by the reaction catalyzed by the xanthine oxidase enzyme upon xanthine. This scavenger effect was also observed when $O_2^{\cdot}$ – was generated by active human neutrophils in which the rate of $O_2^{\cdot}$ – formation was monitored by following the reduction of cytochrome c or the emission of luminol-dependent chemiluminescence. An additional scavenger effect of trimetazidine was measured in an OH. chemical generating system whereby the breakdown of deoxyribose by the thiobarbituric acid assay was detected. This study suggests that trimetazidine might function as an anti-oxy radical compound in conditions of increased oxy radical production [44]. In previous studies, we also showed that the reduction of cardiomyocyte apoptosis is accompanied by an increase in the total activity of SOD and decrease in the content of MDA. Furthermore, results in this investigation show that the apoptotic indices were negatively correlated with the total activity of SOD, and positively correlated with the concentrations of MDA, suggesting that pretreatment with trimetazidine can effectively prevent cardiomyocyte apoptosis, at least in part, via an antioxidant mechanism [10, 11].

### Trimetazidine inhibits neutrophil infiltration and myocardial inflammatory reaction

Reperfusion injury shares many characteristics with inflammatory responses in the myocardium. Neutrophils feature prominently in this inflammatory component of post-ischaemic injury. Ischaemia-reperfusion prompts a release of oxygen free radicals, cytokines and other pro-inflammatory mediators that activate both the neutrophils and the coronary vascular endothelium. Activation of these cell types promotes the expression of adhesion molecules on both the neutrophils and endothelium, which recruits neutrophils to the surface of the endothelium and initiates a specific cascade of cell-cell interactions, leading first to adherence of neutrophils to the vascular endothelium, followed later by trans-endothelial migration and direct interaction with cardiomyocytes. This specific series of events is a prerequisite for the phenotypic expression of reperfusion injury, including endothelial dysfunction, microvascular collapse and blood flow defects, myocardial infarction and apoptosis. Pharmacologic therapy can target the various components in this critical series of events. Effective targets for these pharmacologic agents include: (a) inhibiting the release or accumulation of proinflammatory mediators, (b) altering neutrophil or endothelial cell activation and (c) attenuating adhesion molecule expression on endothelium, neutrophils and myocytes [45]. Interventions that inhibit neutrophil infiltration into myocardial tissue after ischaemia and reperfusion are reported to reduce the size of the infarct [46]. Trimetazidine can protect post-ischaemic hearts from neutrophil-mediated injury. Post-ischaemic impairment of contractile function was significantly attenuated by trimetazidine infusion (10 M, starting 5 minutes before ischaemia and for the initial 15 minutes of reflow). Cardiac oxygen radical production at reflow was also reduced by trimetazidine, independently of direct scavenger effects [47]. Trimetazidine is also a useful drug in preventing inflammatory cardiovascular events after percutaneous

transluminal coronary angioplasty (PTCA). Some authors found that all indirect markers of systemic inflammatory response [tumour necrosis factor-alpha (TNF-alpha), C-reactive protein (CRP) and nitric oxide products (nitrite and nitrate)] were lower in the trimetazidine-treated group than those in the matched control group in the pre- and post-angioplasty periods. Interestingly, in the trimetazidine group, CRP and nitrite levels were significantly lower than in the control group at each time point of the pre- and post-angioplasty periods, but the TNF-alpha levels were significantly decreased only in the post-angioplasty period. Pre-procedural treatment with oral trimetazidine for three days significantly suppressed the elevation of inflammatory markers before and shortly after PTCA [48, 49].

Recent studies have analyzed the impact of trimetazidine on markers of inflammation. Di Napoli et al. [50] found that CRP plasma concentrations remained unchanged in the group of patients receiving trimetazidine, whereas a progressive increase in CRP levels was seen throughout the follow-up period (18 months) in the control group. CRP is a biologic marker widely used to measure the inflammatory status, and the fact that trimetazidine seems to prevent its increase suggests an additional anti-inflammatory activity. Similarly, a decrease in the serum levels of endothelin-1 has been reported in patients with diabetes receiving treatment with trimetazidine, both after short-term (2 weeks) and long-term (6 months) treatment, a decrease not seen in the *placebo* group [51]. Recently, similar findings were reported in a small study involving 15 patients. In this double-blind, randomized crossover trial, a decrease in endothelin-1 release was observed after 15 days of treatment with trimetazidine compared with *placebo* [52]. The mechanisms responsible for these favourable effects of trimetazidine on inflammatory profile and endothelial function are not known; however, they clearly merit further investigation.

In a recent study of isolated rat heart, Di Napoli et al. [53] found that the endothelial nitric oxide synthase (eNOS) mRNA and protein levels were significantly higher in trimetazidine-treated hearts compared to controls. Trimetazidine exerts a significant, nitric oxide-dependent, cardioprotection against ischaemia-reperfusion injury and preserves the endothelial barrier of the coronary circulation. Trimetazidine can also significantly reduce atrial natriuretic peptide mRNA levels compared with untreated rats [54], and decrease plasma brain natriuretic peptide levels in chronic cor pulmonale patients [55]. It has been suggested that atrial natriuretic peptide induced apoptosis in a dose-dependent and cell type-specific manner in neonatal rat cardiac myocytes.

In conclusions, trimetazidine is a clinically effective anti-anginal agent that has no negative inotropic or vasodilator properties. Although it is thought to have direct cytoprotective actions on the myocardium, the mechanism(s) by which this occurs is as yet undefined. The role of trimetazidine in inhibiting cardiomyocyte apoptosis might be one of the important mechanisms.

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